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VITAMIN A
II. Interrelationships Between Pro-Vitamins A and Vitamin A

By W. A. KREHL, Ph.D.

Introduction

Although the role of vitamin A in vision is its only known biochemical site of action, it is known to be concerned with such broad physiological activities as growth, reproduction, and in general the maintenance of the integrity of epithelial tissues.

The fact that vitamin A has not been identified with some specific biochemical reactions at the various places where it functions only emphasizes the magnitude of the task which confronts the nutritional biochemist.

There have been numerous researches concerned with the biological activity of the pro-vitamins A; their conversion to Vitamin A in the body; the standardization and requirements of vitamin A and its pro-vitamins; and the utilization of the various forms of vitamin A. The lack of reference in this review to many important papers does not in any way detract from their value but merely reflects the personal interest of the author.

The Provitamins A

It is well known that plants and microorganisms contain no vitamin A as such, but only carotenoids, some of which are converted to vitamin A when ingested by the animal. Important as vitamin A is to animals, plants either do not require it for similar functions, or they utilize the carotenoids directly. It is certainly clear that plants have a relatively high concentration of these pigments, particularly in the leaves and shoots. Pro-vitamins A would appear to have a definite purpose in the plant kingdom, although their role has not been made clear. It is important to emphasize that the carotenoids are the ultimate precursors of all the vitamin A in nature.

While there may be some pro-vitamins A that have not yet been discovered, there are known nine naturally occurring compounds that have vitamin A activity. These are: α, β, and γ carotene, cryptoxanthene, myxoxanthin, echinenone, leprotene, aphanin and aphanicin. Of these, β carotene is of the greatest importance because it exhibits the greatest activity. Since α andγ carotene and cryptoxanthene are about one-half as active as β-carotene, and since the carotenoid, lycopene shows no activity, it may be concluded that the β-ionone residue is essential for pro-vitamin A activity and, further,

it must not contain additional substituent groups (1). It should be noted that these evaluations are based primarily on growth-promoting properties, since Johnson and Baumann (2) have shown that cryptoxanthene appears to be about as effective as β-carotene for vitamin A storage. In addition to the unsubstituted β-ionone ring the isoprene vitamin A side chain must remain intact to retain full activity.

Stereoisomerism of the carotenoid molecule influences the comparative biological activity of these substances. This has been interpreted to mean that the shape of the molecule determines how well it "fits" to the enzyme carotenase. Just as the right glove fits on the right hand and not on the left, so must the substance on which the enzyme acts "fit or adjust" to the "point of activity" on the enzyme in order for a reaction to take place. Although the naturally occurring carotenoids are the all-trans form, they may exist in a number of stereoisomerism forms (3). In a study with rats (4) the comparative activities of the carotenoids in promoting vitamin A storage in the liver were as follows: setting the value of all-trans β– carotene (natural form) at 100, the values for neo-β carotene B, neo-β carotene U and all trans α carotene were 48, 33 and 25 respectively. In general a trans → cis rotation results in a reduction in the vitamin A activity of the carotenoid and also a general loss of the "straight shape" of the molecule.

Biological Activity of Carotenoids vs. Vitamin A

A study of the literature concerned with the relative biological activity of the carotenoids and vitamin A is difficult because of the influence in the various experiments of numerous variables such as the fat and vitamin E level of the diet. This problem perhaps has its beginning in the fact that in 1934 the Permanent Commission on Biological Standardization of the League of Nations set up the international standard for 1 unit of vitamin A as 0.6 mg. of pure crystalline β-carotene. Since β-carotene is not vitamin A, it was decided to have as a second standard a reference sample of cod liver oil which, after extensive assay, was given a value of 3000 I.U. per gram. The U.S.P. unit in terms of the cod liver oil was to have the same activity as the β-carotene international unit. As it turned out, this reference cod liver oil standard was overvalued and its use in measuring the comparative biological values of materials which contained vitamin A resulted in error and confusion.

The synthesis of vitamin A and its esters soon provided a way out of this dilemma. As the result of extensive collaborative biological tests of vitamin A acetate against β-carotene, the United States Pharmacopeia Vitamin Advisory Board announced a solution of vitamin A acetate as their new reference preparation to replace the old cod liver oil standard. The amount

of vitamin A acetate found equivalent to 1 I.U. (0.6 ug.) of β-carotene was 0.344 ug. (or 0.3 ug. of vitamin A alcohol), so that 1 gm. of pure vitamin A must contain 3.3 million I.U. The conversion factor for vitamin A acetate which relates the spectroscopic readings (physical method) to the results of biological tests expressed in international units is 1900. It must be concluded from these extensive collaborative researches and biological tests that 0.3 ug. of vitamin A has the same activity of 0.6 ug. of β-carotene and that the molecule of β-carotene yields biologically only 1 molecule of vitamin A.

That there is not complete agreement on this conclusion, is indicated by the work of Koehn (5) who compared the relative biological activities of vitamin A, vitamin A acetate, and pure β-carotene under identical conditions which provided an adequate level of α-tocopherol (1 mg./rat/day). These results showed that β-carotene and vitamin A had equal potency on a weight basis. In an extension of this type of study Burns *et al* (6) found that only at a level of 1 mg. of α-tocopherol per rat per day did β-carotene have the same activity as vitamin A. Although these workers found that the relative activities of vitamin A and β-carotene were not changed when fat was removed from the diet, the magnitude of the growth response was less on the low fat ration.

A striking observation was noted when as little as 0.16 to 0.32 per cent of mineral oil was added to the diet. The efficiency of utilization of β-carotene was markedly decreased. Vitamin A was much less affected. This would seem to be a serious objection to the use of mineral oil in natural feeds to minimize dustiness.

It has also been reported by Russell *et al* (7) that in chickens, carotene absorption was correlated with the dietary level of fat, whereas the absorption of vitamin A was not as greatly affected by dietary fat levels.

One fact might be deduced from these and other results, namely that extraneous factors have a greater bearing on the optimum biological potency of β-carotene than vitamin A. To illustrate further, Kemmerer *et al* (8) have shown that "xanthophylls and chlorophylls reduce the utilization of carotene for liver storage of vitamin A about 20 percent." These authors point out that the xanthophylls may in part account for the low utilization of carotene in many vegetables.

Differences in evaluating biological potency may also arise when tissue storage or growth is the basis of assay. The levels of carotenoid pigments needed to allow detectable storage of vitamin A are about 10 times as much as the amount needed to prevent symptoms of avitaminosis and stimulate the growth response.

Site of Conversion of Carotene to Vitamin A

Since the liver is the chief organ of storage for vitamin A and is the center of considerable metabolic activity, it is not strange that the liver was considered to be the place where the body converted carotene into vitamin A. Although this idea persisted until only recently, certain objections had been raised, namely that no carotene could be found in the blood of certain animals even after a carotene meal. Furthermore, reports on the effectiveness of parenteral carotenes were conflicting until in 1946 Sexton *et al* (9) showed conclusively that parenterally administered carotene in vitamin A deficient rats was ineffective in correcting the deficiency although it was accumulated in the liver. From these results, Sexton suggested that "a possible site for the transformation of carotene to vitamin A might be in the intestinal wall."

Credit for establishing the intestine as the site of conversion of carotene to vitamin A must be given to workers in several laboratories (10, 11, 12, 13).

Although the details of the experiments can be found in the original papers the chief findings are as follows. It is clear that the liver and not the intestine is the chief storage site for vitamin A since the latter can be depleted of vitamin A in a few days even in animals with large liver reserves. If such animals are given carotene, vitamin A promptly appears in the small intestine. It is interesting that the appearance of the vitamin seems to be confined to the small intestine and does not appear in that part of the intestine preceding the entrance of the common bile duct. In the experiments with deficient animals, vitamin A is found first in the small intestine before any other site and for a time exceeds the accumulation in the liver.

It is interesting that the esterified form of vitamin A is found in the intestine whether carotene, vitamin A alcohol or acetate, or retinene are fed. All seem to lead to a maximum concentration of vitamin A ester in the intestinal wall.

The lymph is an important avenue of departure for vitamin A ester from the intestinal wall. In fact, it was shown (14) that by tapping and removing the lymph flowing from the intestine, vitamin A was prevented from reaching the blood or liver.

Also ruling out the liver as a site of conversion of carotene to vitamin A is an experiment by Krause and Pierce (15) who in a two stage operation, blocked the liver circulation by closing off the portal vein, the hepatic artery and the common bile duct after which the rats were given orally an aqueous dispersion of carotene in Tween 40 and water. Blood samples taken 6 or 8 hours after feeding the carotene showed a large increase in vitamin A.

Thus it must be concluded that carotene is transformed into vitamin A in the wall of the small intestine. The exact enzymatic mechanism remains to be shown. It is evident that because the intestine serves this important converting function any impairment of the integrity of the intestinal wall by either diet, dietary deficiency of other factors, or such changes as may be induced by excessive ingestion of antibiotics, may seriously interfere with the biological potency of the precursors of vitamin A.

Requirement of Vitamin A

Consideration of the requirement of a vitamin brings to mind the definition given for the dosage of a drug by a famous teacher of pharmacology — "The dosage of a drug is *enough,* but *not too much.*" When questioned further as to how *enough* was determined the answer was "through knowledge and experience." The important aspect of this definition for the nutritionist is the implied but unspoken concept that dosage or requirement is *variable* and can be influenced up or down by extraneous factors. The ideal diet is one that is so "balanced" that the requirement for each and all of the individual nutrients, not only vitamins, is minimal for optimal physiological performance. To illustrate this point outside the field of vitamins, the ratio between calcium and phosphorus is a much more important consideration than the absolute amount of either mineral.

So too, factors such as the vitamin E content of the diet, the absence of rancid fat in the diet, *et cetera,* influence to a significant degree the actual requirement for vitamin A.

In general, the human requirement of a vitamin is arrived at by an extension of the results obtained with experimental animals. In the case of vitamin A however we have as a basis for a requirement figure a large-scale human experiment. The details of this extensive and valuable research are to be found in a Medical Research Council report (15a) published in England.

The experiment was conducted in Great Britain on a group of conscientious objectors who were placed on vitamin A deficient diets. Curiously, very few of the subjects in this study ever became depleted enough to show significant change as the result of dosage with vitamin A or carotene. "No unequivocal signs of depletion appeared within the first year." It appears that the liver storage of vitamin A may be substantial and may sustain the individual for relatively long periods of time.

As a result of this and other results, the minimum daily protective dose of vitamin A for a healthy adult was considered to be 1300 I.U. by authorities in England. To allow for individual variation and to provide a margin of safety, 2500 I.U. of vitamin A was suggested as the daily adult requirement.

The requirement for β-carotene is even more difficult to assess because of the many complicating factors indicated above. Assuming that all the β-carotene given is absorbed, the minimum requirement for a healthy adult was considered to be 1500 I.U. (It will be remembered that the I.U. is 0.6 ug. of β-carotene or 0.344 ug. of vitamin A acetate = 0.3 ug. of vitamin A). Again to provide the safety margin this figure was doubled to give a value of 3000 I.U.

It was also shown in these vitamin A studies with human subjects that in order to insure that the body actually absorbs 3000 I.U. of β-carotene a much larger and variable amount of the carotenoid had to be provided by the diet. Because many factors influence the "absorbability and availability" of carotene it is very difficult and even undesirable to state a specific daily requirement figure for β-carotene. With some reluctance therefore a value of 7500 I.U. of β-carotene (3 times the vitamin A value) was put forward.

The recommended dietary allowance of vitamin A (not to be confused with requirement) in the United States is placed at 5000 I.U. per day. This allowance also takes into account that "approximately two-thirds of the vitamin A value of the average diet in this country is contributed by carotene and that carotene has half or less than half the value of vitamin A."

Another factor which may complicate the carotene requirement in certain animals is seen in the report by McGillivray (16) that the intestinal flora of sheep may synthesize considerable amounts of carotene. It has also been shown by Barrick *et al* (17) that though no absorption of carotene takes place from the cecum or colon there may be absorption from lower regions of the ileum which is the area where McGillivray reports carotene synthesis to occur.

Utilization of Vitamin A

It has been indicated above that many factors influence the utilization and biological value of vitamin A and particularly its pro-vitamins. Vitamin A, as a fat soluble vitamin, has its absorption influenced by such important factors as the level of dietary fat, the chemical form of the vitamin *(i.e.* free alcohol or esters) and the presence of bile. A new factor worthy of consideration is the use of emulsifying and dispersing agents such as the Tweens* which have made possible the preparation of aqueous dispersions of fat soluble materials that behave in many ways like water solutions. It has been shown by Kramer *et al* (18) that the rate of absorption of vitamin A from these aqueous dispersions is significantly greater than from fish liver oil alone in normal children as well as those with celiac disease. In an extension

*Polyoxyethylene derivatives of partial esters of fatty acids and di-anhydides of the hexitols, sorbitol and mannitol.

of this study, Sobel *et al* (19) has shown that the increased vitamin A blood levels following dosage with the aqueous dispersions of vitamin A are a true reflection of increased absorption, since from a known dosage of vitamin A, given in either oil or aqueous dispersion, the liver storage values of A were much higher in rats that had received the vitamin as an aqueous dispersion. Numerous subsequent reports in the literature provide ample confirmation of these observations.

Although the various vitamin A esters both natural and synthetic are hydrolized before vitamin A is absorbed, the extent to which this takes place, the factors influencing the rate of hydrolysis, and the role that these factors have on the utilization of vitamin A has not been completely established on a quantitative basis.

In an investigation of the relative biologic potency of various forms of vitamin A in humans, Week and Sevigne (20) showed that vitamin A alcohol allowed greater increases in serum vitamin A levels than any other form of the vitamin, and vitamin A acetate was similarly superior to the natural esters of vitamin A. Although the differences in effectiveness between the various forms of vitamin A under the conditions of these experiments were statistically significant they were all well utilized and from a practical point of view, the differences were not great.

Week and Sevigne (21) have also determined the effect of such vitamin A diluting agents as ethyl laurate, corn oil and mineral oil on the utilization of various forms of the vitamin by the rat. Ethyl laurate as a diluting agent resulted in poor utilization of both vitamin A alcohol and its esters; mineral oil reduced the utilization of vitamin A esters more than the free alcohol; corn oil on the other hand allowed the best utilization and storage of the various forms of the vitamin. In another experiment, where the intake of the vitamin was greatly reduced, corn oil allowed superior utilization of the natural vitamin A esters as compared to the free alcohol or vitamin A acetate. Mineral oil was inferior for all forms of the vitamin.

Again this emphasizes what was said earlier — that the entire composition of the diet has a significant effect on the nutritive capacity or potentiality of each individual component — the nutritionist must be ever cognizant of this.

Distribution of Vitamin A and Carotene

Fish liver oils and mammalian livers provide the best natural sources of vitamin A, Table 1, while the fish-body oils and the fat of animals such as pork, beef, and mutton are poor "carriers" of this vitamin.

TABLE 1—Vitamin A Content of Some Official Preparations.

Preparation	*I.U. Vitamin A per Gram*
Cod liver oil	850
Cod liver oil concentrates	50,000–60,000
Halibut liver oil	60,000

As has been stated above, preformed vitamin A does not occur as such in plants. Most leafy vegetables are, however, rich in pro-vitamin A, particularly β-carotene. Illustrations of some of the best sources of pro-vitamin A from the plant kingdom are seen as follows:

TABLE 2—Dietary Sources* of Vitamin A.

(Values are given in I.U. of vitamin A per 100 g. of edible portion.)

Asparagus	1000	Kale	7540
Beet Greens	6700	Spinach	9420
Broccoli	3500	Squash (Winter)	4950
Carrots	12000	Sweet Potatoes	7700
Collards	6870	Turnip Greens	9540

*Abstracted from U.S.D.A. Misc. Pub. No. 572; "Tables of Food Composition in Terms of Eleven Nutrients" (1945).

Although red-palm oil has a very high carotene content, most other vegetable oils are practically devoid of vitamin A activity.

Dairy products are both excellent and practical sources of vitamin A activity and are distinctive in that this activity is derived partly from vitamin A itself and partly from carotene. Margarines that are fortified with adequate amounts of vitamin A or carotene are, of course, also good sources of this vitamin.

Conclusion

Although only a few of many outstanding researches have been reviewed here, all of the available evidence indicates that the vitamin A content of the organism is influenced by many factors, only one of which is the actual dietary intake of the vitamin.

For the future, a number of important areas of research need to be developed. The protective action of substantial reserves of vitamin A in the liver against metabolic stress needs considerable study and evaluation. It is also of importance to study the factors which cause the disappearance from the body of large amounts of vitamin A under both normal and pathological

conditions. This raises the question as to the nature of the metabolic products of vitamin A degradation.

We know that the animal ingests and utilizes both vitamin A preformed and the pro-vitamin. The chemical structure of the carotenoid in both direct and subtle ways determines whether it will be converted to vitamin A and how efficiently. This conversion has been shown to take place in the wall of the small intestine rather than in the liver as earlier supposed. In the intestinal wall the vitamin A is esterified and transported, largely by the lymphatics, to the blood and eventually to the liver. In the liver the vitamin A is stored primarily as the ester form of the vitamin. Free vitamin A is maintained in the blood at a reasonably constant level of about 30 ug. per 100 ml. of plasma by the action of esterase on stored vitamin A esters. Vitamin A is distributed by the blood to the various sites at which it exerts its action. The eye appears to be the one location in which a biochemical role has been reasonably well elucidated.

As in all research, each bit of information, even though insignificant itself, broadens the scope of knowledge and permits a greater appreciation of the vast intricacies of biologic function.

REFERENCES CITED

1. GOODWIN, T. W., *Br. J. of Nutrition 5,* 94 (1951).
2. JOHNSON, R. M., and Baumann, C. A., *Arch. Biochem., 19,* 493 (1948).
3. ZECHMEISTER, L., *Chem. Rev. 34,* 267 (1944).
4. JOHNSON, R. M., and Baumann, C. A., *Arch. Biochem., 14,* 361 (1947).
5. KOEHN, C. J., *Arch. Biochem. 17,* 337 (1948).
6. BURNS, M. J., Hauge, S. M., and Quackenbush, F. W., *Arch. Biochem. 30,* 341, 347 (1951).
7. RUSSELL, W. C., Taylor, M. W., Walker, H. A., and Polskin, L. J., *J. Nutrition 24,* 199 (1942).
8. KEMMERER, A. R., Fraps, G. S., and DeMottier, J., *Arch. Biochem. 12,* 135 (1947).
9. SEXTON, E. L., Mehl, J. W., and Deuel, H. J., *J. Nutrition 31,* 299 (1946).
10. GLOVER, J., Goodwin, T. W., and Morton, R. A., *Biochem. J. 41,* XLV (1947).
11. MATTSON, F. H., Mehl, J. W., and Deuel, H. J. Jr., *Arch. Biochem. 15,* 65 (1947).
12. WIESE, C. E., Mehl, J. W., and Deuel, H. J. Jr., *Arch. Biochem. 15,* 75 (1947).
13. THOMPSON, S. Y., Ganguly, J. and Kon, S. K., *Brit. J. Nutrition* 1, V (1947).
14. THOMPSON, S. Y., Braude, R., Coates, M. E., Cowie, A. T., Ganguly, J., and Kon, S. K., *Brit. J. Nutrition 4,* 398 (1950).
15. KRAUSE, R. F., and Pierce, H. B., *Arch. Biochem. 19,* 145 (1948).
15a. HUME, E. M., and Krebs, H. A., *Spec. Rep. Ser. Med. Res. Coun.,* London, No. 264.
16. MCGILLIVRAY, W. A., *Brit. J. Nutrition 5,* 223 (1951).
17. BARRICK, E. R., Andrews, F. N., and Bullard, J. F., *J. Animal Sci. 7,* 539 (1948).
18. KRAMER, B., Sobel, A. E., and Gottfried, S. P., *Am. J. Dis. Child. 73,* 543 (1947).
19. SOBEL, A. E., Sherman, M., Lichtblau, J., Snow, S., and Kramer, B., *J. Nutrition 35,* 225 (1948).
20. WEEK, E. F., and Sevigne, F. J., *J. Nutrition 40,* 563 (1950).
21. WEEK, E. F., and Sevigne, F. J., *J. Nutrition 42,* 525 (1950).

NEWS DIGEST

Diet and Weight Reduction — Obesity is the direct result of continued caloric intake in excess of caloric output, and is represented by an accumulation of an abnormal amount of adipose tissue. The increased incidence of obesity in this country has been cited by many as an important factor in the general increase in the so-called degenerative diseases, such as hypertension and diabetes. Although the fat man is usually represented as a jovial happy creature, the psychologist suggests that eating food is an outlet — "to relieve the tensions of emotiona! deprivations. Worry, fear, boredom and anxiety undoubtedly result in overeating for certain individuals." The exact physiological mechanisms which cause hyperphagia are not known but are thought to be related to control by the hypothalmus.

Just as excesses in caloric intake produce obesity, weight loss must inevitably accompany restriction of caloric intake to a level less than the caloric output. To tell an obese person that all he has to do to lose weight is to "eat less" is about as valuable a pearl of wisdom as the fight manager's words — "Keep punching" — to his beaten boxer.

How to reduce caloric intake so as to lose weight and maintain good health and disposition is really the crux of the problem of proper weight reduction. What is the role of the composition of the diet in this question? Many popular magazines have published articles on weight-reducing diets. Most of these require radical and costly departures from the usual dietary patterns of the average family and are therefore difficult to adopt. Relatively few objective studies have been made to "explore the problems of weight loss from the viewpoint of developing more satisfactory dietary procedures than those now in common use." Such a study has been reported in two papers by Cederquist *et al* and Brewer *et al* in the *J. of the Am. Dietetic Association, Vol. 28,* pp. 113-116 and pp. 213-216, (1952). The subjects chosen for the two investigations were college girls who were from 8 to 41 percent overweight judged by anthropometric measurements. The basic plan of the experiment allowed a free selection of food for a period of two weeks. The last 7 days of the self-selected regimen were taken as the first balance period, after which the girls were placed in Series I on a low-fat reducing diet which supplied about 1200 calories and 10.5 gm. of nitrogen per day. Analyses were made of the food for calories and nitrogen and at routine intervals urine and fecal collections were made and analyzed for nitrogen; in addition the caloric content of the feces was determined. Activity records were also kept so that a reasonably accurate knowledge of both caloric intake and output was available. Although a daily caloric deficit of 800-1000 calories on the low-fat reducing diet was noted, the weight losses were surprisingly small. In addition the "subjects reported lack of pep throughout the study. They were discouraged because they were always conscious of being hungry."

The second series of subjects studied were given a more liberal caloric intake of 1500 calories per day which provided 16 gm. of nitrogen and about 85 to 90 gm. of carbohydrate per day. More than half of the ingested calories of this diet were derived from fat and it was termed the low-carbohydrate reducing diet. "The weight losses which were obtained after 16 weeks on this experimental diet ranged from 8.8 to 16.8 kg." "Without excep-

tion, the low-carbohydrate reducing diet resulted in satisfactory weight losses when the subjects adhered to the diet. The subjects reported a feeling of well-being and satisfaction. Hunger between meals was not a problem."

Maintenance of weight after reduction could be achieved by increasing such foods of the basic menu as milk, cereal foods, fruits, and vegetables. Simple desserts were added on the basis of preference to bring the subject to approximately caloric balance.

Careful observations were made during these investigations on the utilization of nitrogen and calcium "during a period of unrestricted food intake, and during and after weight reduction." To repeat, the low-fat diet supplied 1200 calories and 10.5 gm. of nitrogen per day, while the low-carbohydrate diet provided 1500 calories and 16.2 gm. of nitrogen per day. Weight loss was effected more readily and with much less discomfort on the latter diet.

"The mean nitrogen retentions for subjects on the low-fat diet ranged from 0.21 to 2.84 gm. per day; the mean nitrogen retentions for subjects on the low carbohydrate diet ranged from —0.74 to 1.29 gm. per day."

Although calcium retention by subjects on the weight reducing diets "was significantly lower than that of women of average weight on controlled diets, (it) did not differ significantly from values reported for college women on self-selected diets."

These studies present some very interesting results and reflect the kind of intelligent approach to the problem of weight reduction that the nutritionist would like to recommend for all to consider.

Carbonate is Utilized in the Synthesis of Milk Constituents in experiments conducted by Kleiber *et al. J. Biol. Chem. 195,* 707 (1952). Lactating cows were given labeled CO_2 as sodium bicarbonate which contained C^{14}. According to the specific activities of the isolated fat, casein, and lactose, it was evident that the carbon had come from carbonate to the extent of 2, 4, and 10 percents respectively. In a similar type of experiment, R. J. Block and J. A. Stekol. *Proc. Soc. Expt'l. Biol. and Med. 73,* 391 (1950), fed radio-active sulfur as sodium sulfate to a cow after which milk was collected for several days. The proteins isolated from this milk showed considerable radio-activity and when they were hydrolyzed, cystine and methionine were separated by paper chromatography and both were found to contain radio-active sulfur. These techniques should point the way to new fields of research and be of great value as tools for studying the rates of synthesis of the various components of milk.

Raw Soybeans May Produce Vitamin A Deficiency according to a number of reports. Shaw *et al, J. Dairy Sci. 34,* 176, (1951) found that calves fed a raw soybean test diet had significantly lowered plasma vitamin A and carotene values. Although no explanation is offered to explain the effect of raw soy beans, their use may be dangerous especially if the cattle are given roughage of low carotene content.

Ultraviolet Light Affects the Proteins of Milk Serum under circumstances of prolonged exposure (20 sec.), but little change is noted after exposure under practical conditions for short periods of time. Changes in the proteins were determined by examination of the electrophoretic patterns of the milk serum proteins. No change in serum proteins

would result from the short (0.5 sec.) irradiation of milk to be used as a source of vitamin D. It should be noted, that in the United States, the use of ultraviolet light for increasing the vitamin D content of milk has been almost completely, if not entirely, supplanted by the use of vitamin D concentrates. Only by so doing has it proved possible to get the vitamin D concentration required by government standards without adversely affecting the flavor and other properties of milk. — Hellbrugge *et al., Ztschr. Kinderheilk 70,* 1 (1951).

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